Relationship Between Steady-State Pharmacokinetics and Hemodynamic Effects of Inhaled Isobutyl Nitrite in Conscious Rats

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ABSTRACT Our objective was to examine the pharmacokinetic/hemodynamic properties of inhaled isobutyl nitrite (ISBN) in rats. ISBN is one of the volatile organic nitrites that has been used primarily as a drug of abuse. Recent studies indicate, however, that these compounds may be superior to organic nitrates for cardiovascular use because they do not produce vascular tolerance. Rats inhaled ISBN over an exposure range of 20 to 1200 ppm for 1 hour. The effects of ISBN on blood pressure and heart rate were determined and blood concentrations of ISBN were analyzed with use of gas chromatography. Apparent steady-state blood levels of ISBN were achieved during inhalation and were linear with exposure concentration (blood concentration: 0.05 to 3.5 μM; exposure concentration: 23 to 1177 ppm; $r^2 = 0.92$). Inhaled ISBN caused rapid, dose-dependent, and parallel reductions in systolic and diastolic pressure, while heart rate increased maximally to 22%. A sigmoid E_{max} model could describe the mean arterial pressure effect of inhaled ISBN ($E_{max} = 55\%$; $EC_{50} = 0.51$ μM). After inhalation, blood pressure and heart rate quickly returned to baseline, without any withdrawal rebound effect. Inhaled ISBN produced a rapid onset of action on heart rate and blood pressure, and these effects were sustained over 60 minutes of exposure. Abrupt drug withdrawal did not lead to hemodynamic rebound. The blood pressure effects were related to ISBN blood concentration by the sigmoid E_{max} model. These results provide new information on the pharmacokinetic / pharmacodynamic relationship of a representative nitrite inhalant.

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INTRODUCTION

Organic nitrites, such as isobutyl nitrate (ISBN) and isoamyl nitrite (ISAN), are volatile esters of nitrous acid that were introduced during the 1800s as therapeutic agents for relief from acute attacks of angina pectoris because they could abate the discomfort associated with this disease by lowering arterial tension. ISAN is approved in the United States as an anti-anginal agent, but it is seldom used in the clinic because of its short duration of action and volatile properties. Unlike ISAN, ISBN is not marketed as an anti-anginal compound, even though the pharmacological effects are similar. ISAN is also used in evaluation of heart murmurs and coronary artery disease and as an antidote for cyanide toxicity.

At present, organic nitrites receive greater notoriety for their recreational use than for their therapeutic application. These agents have become popular street drugs used primarily to alter consciousness, enhance meditation, and intensify sexual experiences. In humans, deep inhalations of nitrites have been reported to produce nonspecific smooth muscle relaxation, causing hypotension and cutaneous flushing, sometimes followed by reflex vasoconstriction and tachycardia. Individuals who abuse nitrites expose themselves to extremely high doses, estimated to exceed 1500 ppm at a time (1). Deep inhalations can produce euphoric feelings that last about 5 minutes, which is most likely due to dilation of the cerebral vasculature. Frequent abuse of nitrites has been correlated with seropositivity to human immunodeficiency virus in a number of epidemiological studies [2-;4]. Nitrite abuse was found to be a common factor in almost every reported case of Kaposi's Sarcoma (KS), in that AIDS patients with KS were more likely to have a history of nitrite abuse than AIDS patients with other complications, such as pneumocystis pneumonia [5-;8]. Although it

has not been proven that nitrite abuse contributes to KS, it has been shown that chronic exposure to a nitrite inhalant is immunosuppressive in mice (9-11).

The vascular action of organic nitrites is mediated by the release of nitric oxide (NO), which can activate soluble guanylate cyclase to cause vascular relaxation (12). All NO donors, such as organic nitrates (nitroglycerin, NTG) and sodium nitroprusside (SNP), share this common biochemical pathway. However, we have shown that the metabolic (13) and hemodynamic (14) properties of organic nitrites are different from those of organic nitrates, suggesting that the pharmacokinetic (PK) and pharmacodynamic (PD) relationships of organic nitrites might also be different from those of organic nitrates (15-17). No PK/PD information is available for the organic nitrites, in spite of their use over more than a century.

Our objective, therefore, was to characterize the PK/PD properties of ISBN, a representative inhalant nitrite. Our aims were (1) to determine the effect of inhaled ISBN on blood pressure and heart rate and (2) to describe the relationship between ISBN blood levels and mean arterial pressure (MAP) in conscious rats. This report presents the first detailed pharmacokinetic/hemodynamic characterization of a nitrite inhalant.

MATERIALS AND METHODS

Materials

ISBN was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Pentane was of capillary GC-GC/MS solvent grade (Burdick and Jackson, Muskegon, MI).

Animal Preparation

Male Sprague Dawley rats of 300 to 350 g were used in the study. For the pharmacokinetic/hemodynamic studies, rats were anesthetized with ketamine/xylazine (90/9 mg/kg; IM), and cannulae (PE-50; i.d. 0.76 mm) were implanted via the left femoral artery and right carotid artery for blood withdrawal and hemodynamic measurements, respectively. For studies in which blood samples were not taken, rats were cannulated via the right carotid artery only.

Experiments were performed on conscious rats at least 24 hours after placement of the catheters. On the day of the experiment, each animal was placed in an inhalation chamber (Braintree Scientific, Braintree, MA), into which ISBN was introduced with a vaporizer (Cyprane Ltd., Keighley, England) calibrated for ISBN and medical grade air (Strate Welding, Buffalo, NY), as previously described (18). A carotid artery catheter was connected to a RS3400 Gould physiograph (Gould, Cleveland, OH) or a Cardiomax II® instrument (Columbus Instruments, Columbus, OH) via a Statham pressure transducer (model P23 ID; Statham, Murray Hill, NJ) for the recording of blood pressure and heart rate.

Study Protocol

We assessed the hemodynamic effects of inhaled ISBN (20 to 1200 ppm) during and after 60 minutes of exposure. ISBN chamber concentrations were monitored as described below. Blood samples were withdrawn for ISBN analysis at 20, 40, and 60 minutes following onset of inhalation.

Analysis of ISBN

The details of the assay procedure for the analysis of ISBN in rat blood have been reported elsewhere (18). Briefly, blood samples containing ISBN (0.4 mL) were taken through a heparinized cannula with a 0.5-mL gas-tight glass syringe stored at 0°C just prior to sampling. The blood was immediately processed under ice-cold gas-tight conditions by vortexing with an equal volume of pentane (0°C) containing the internal standard, n-propyl nitrate. An aliquot of pentane was subsequently removed and placed in a sealed glass vial for injection. Samples were injected (3 μL) onto a 5890A gas chromatograph (Hewlett Packard, Avondale, PA) equipped with an electron capture detector and a J&W Scientific (Folsom, CA) DB-1 capillary column (30 m length; 0.32 mm i.d.; 1 μm film thickness). The samples were chromatographed with use of gradient temperature elution: 30°C for the initial 9.5 minutes followed by 45°C for the remaining 8.5 minutes. The oven

temperature was increased at a rate of 60°C per minute. The total run time was 18.3 minutes. Extra-dry grade nitrogen (Strate Welding, Buffalo, NY) was used both as the carrier gas (1.0 mL/minute) and the detector gas (21.0 mL/minute). The injector and detector temperatures were set at 45°C and 195°C, respectively. As we previously reported (18), the intra- and interassay variabilities were less than 11% and 15%, respectively, over a concentration range of 9.7 nM to 3.9×10^3 nM ISBN in blood.

During the experiments, volatized ISBN in the air of the inhalation chamber was analyzed with use of a gas chromatograph with flame ionization detection. Briefly, 1.0 mL was withdrawn from the chamber with a gas-tight syringe followed by injection of a 30-µL aliquot on the gas chromatograph. ISBN concentrations in the inhalation chamber were determined 3 times during an exposure interval and are reported as the mean ± standard deviation. Calibration standards ranging from 0 to 2046 ppm were prepared by injecting microliter volumes of ISBN into gas-tight vials that were allowed to vaporize before analysis. Inter- and intra-day variabilities of the assay procedure for ISBN were less than 13%.

Pharmacodynamic Data Analysis

The sigmoid E_{max} model was used to describe the relationship between ISBN blood concentration and mean arterial pressure (MAP) by applying the equation $E = (E_{MAX} C_{ISBN}^{n}) / (EC_{50}^{n} + C_{ISBN}^{n})$. In this relationship, E is the observed effect (% decrease in MAP), E_{max} is the maximal effect, EC_{50} is the concentration at half the maximal effect, and n is the power function describing the slope of the curve. MAP was determined by the equation MAP = DP + 1/3 (SP-DP), where SP is the systolic pressure and DP is the diastolic pressure. Heart rate was calculated directly by the Cardiomax II® instrument and recorded.

Pharmacodynamic data were expressed as mean ± standard deviation from 3 to 4 rats per exposure concentration of ISBN. Statistical analyses were performed via one-way analysis of variance to compare the effect of inhaled ISBN on heart rate and blood pressure versus baseline (pre-exposure values) following different exposure concentrations of ISBN. *P* values greater than 0.05 were considered not significant. The pharmacodynamic data were analyzed with ADAPT II software (Biomedical Simulations Resource, Los Angeles, CA).

RESULTS

Hemodynamics of Inhaled ISBN

As an illustration, the blood pressure effects after ISBN inhalation at 3 select exposure concentrations, viz., 54 ± 6, 301 ± 9, and 1177 ± 47 ppm, are shown in Figure 1.

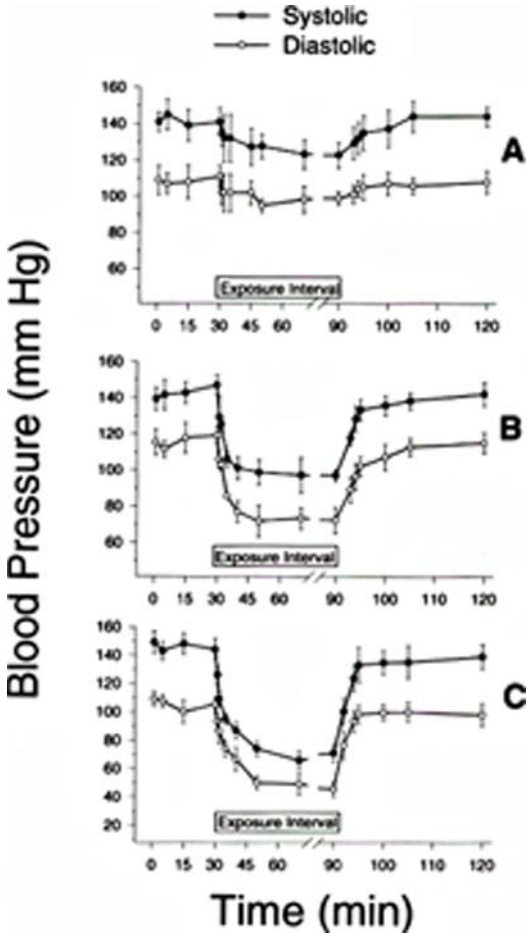

Figure 1. Systolic and diastolic blood pressure versus time following inhaled ISBN at mean exposure concentrations of 54 (A), 301 (B), and 1177 (C) ppm for 60 minutes. The symbols represent the mean observed values and the error bars represent the standard deviation of mean observations from 3 to 4 rats.

Both systolic and diastolic blood pressures decreased rapidly upon ISBN inhalation, and these effects plateaued around 10 minutes after drug administration. The extent of reduction in systolic and diastolic blood pressure was 9.5 ± 4.1 and 7.2 ± 3.6% for rats exposed to a mean exposure concentration of 54 ppm ISBN (Figure 1A), 33.7 ± 4.4 and 37.0 ± 7.3% for 301 ppm (Figure 1B), and 50.7 ± 5.3 and 50.4 ± 5.1% for 1177 ppm (Figure 1C), respectively. Withdrawal of ISBN after 60 minutes of exposure led to rapid reversal of both systolic and diastolic blood pressures to baseline values within 10 minutes.

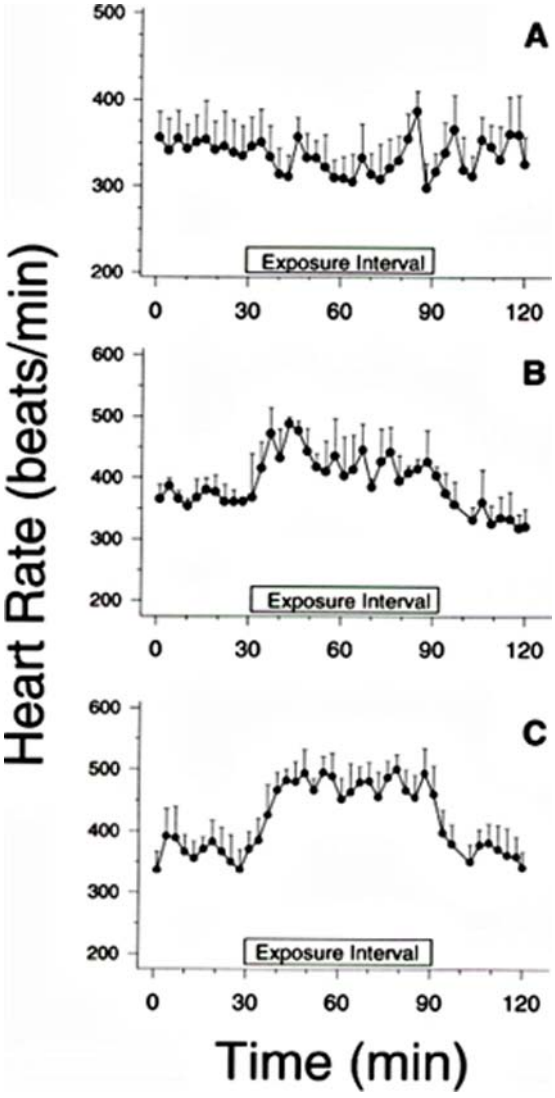

Figure 2. Changes in heart rate versus time following inhaled ISBN at mean exposure concentrations of 54 (A), 301 (B), and 1177 (C) ppm for 60 minutes. The symbols represent the mean observed values and the error bars represent the standard deviation of mean observations from 3 to 4 rats.

The corresponding changes in heart rate at these exposure concentrations are shown in Figure 2. Up to a mean exposure concentration of 54 ppm ISBN (Figure 2A), heart rate did not appear to change from baseline values ($P > 0.05$). However, rats inhaling higher levels of ISBN exhibited pronounced increases in their heart rates. For the exposure of ISBN concentrations of 301 ppm (Figure 2B) and 1177 ppm (Figure 2C), the heart rate increased to 426 ± 39.7 beats/minute ($P < 0.05$ vs. baseline) and 464 ± 34.9 beats/minute ($P < 0.01$ vs. baseline), respectively. Upon termination of the exposure, heart rates returned to baseline within 10 to 15 minutes.

We also examined changes in blood pressure during ISBN administration over 60 minutes at 7 different exposure concentrations, 23 ± 3, 54 ± 6, 102 ± 11, 301 ± 9, 511 ± 25, 890 ± 31, and 1177 ± 47 ppm ISBN. At an average exposure concentration of 23 ppm, there was little effect of ISBN on blood pressure ($P > 0.05$ versus baseline); however, higher levels of exposure led to more pronounced steady-state decreases in both systolic and diastolic blood pressures within 15 to 20 minutes. Figure 3 shows the maximum decrease in systolic and diastolic blood pressure, calculated as the average value of those recorded after 20, 40, and 60 minutes of exposure for each rat, as a function of ISBN exposure concentration.

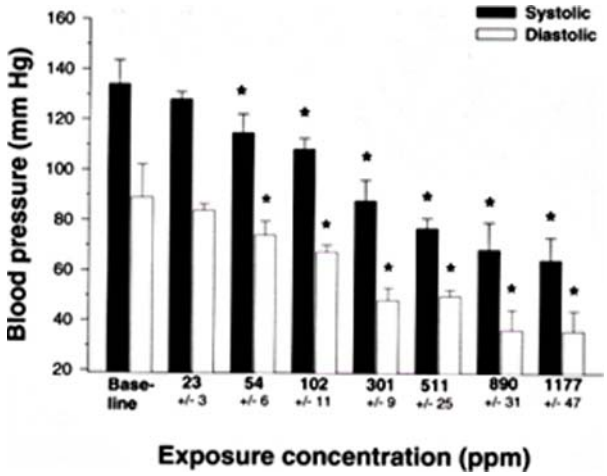

Figure 3. Magnitude of decrease in systolic and diastolic blood pressure at different mean ISBN exposure concentrations. The symbols represent the mean observed values and the error bars represent the standard deviation of mean observations from rats (baseline, n = 10; ISBN exposed, n = 3 to 4; *$P < 0.05$ vs baseline).

It is evident that both systolic and diastolic pressures decreased dose-dependently in parallel with increasing concentrations of inhaled ISBN. Maximal hemodynamic effects of ISBN were achieved beyond 890 ppm.

Steady-State Pharmacokinetics of Inhaled ISBN

Blood concentrations following exposure of ISBN are shown in Figure 4A and 4B.

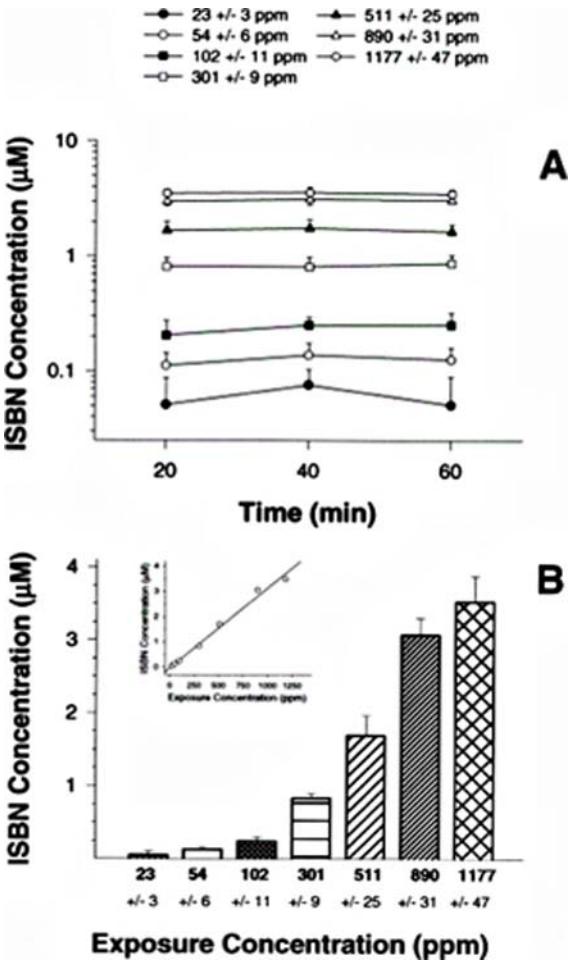

Figure 4. Blood concentration of ISBN versus time (A) and mean exposure concentration (B) during inhalation of ISBN. In B, inset shows linearity of blood concentration with ISBN exposure level. The symbols represent the mean observed values and the error bars represent the standard deviation of mean observations from 3-4 rats.

Apparent steady-state blood levels of ISBN were achieved during exposure (Figure 4A) and were proportional to exposure concentration (Figure 4B). Mean exposure concentrations of 23, 54, 102, 301, 511, 890, and 1177 ppm ISBN produced apparent steady-state blood concentrations of 0.05 ± 0.03, 0.13 ± 0.03, 0.24 ± 0.06, 0.83 ± 0.16, 1.69 ± 0.27, 3.07 ± 0.23, and 3.53 ± 0.35 μM, respectively.

The relationship between ISBN blood concentration and hemodynamic profile for all animals is shown in Figure 5.

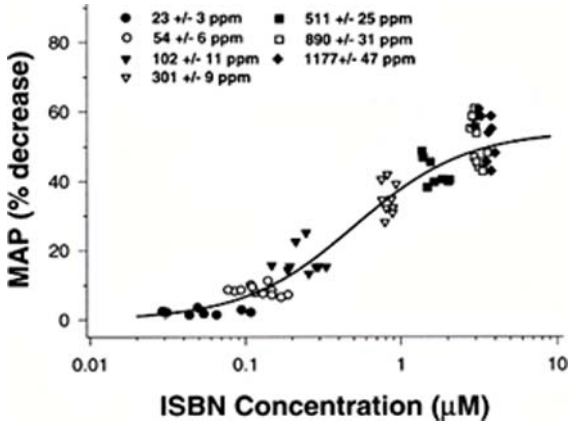

Figure 5. Relationship between MAP and ISBN blood concentration over different exposure levels of ISBN. The solid line represents the fit of the individual data points to the sigmoid E_{max} model.

A sigmoid E_{max} model was used to describe the MAP effect of inhaled ISBN. Parameter estimates (% CV) of E_{max}, EC_{50}, and the Hill coefficient were 54 (5.1)% decrease, 0.51 (12.2) μM ISBN, and 1.2 (4.9), respectively.

DISCUSSION

Despite a general understanding of the cardiovascular effects of nitrites, their pharmacokinetic / hemodynamic relationship is unknown. In the present study, we investigated the relationship between ISBN blood concentration and inhaled dose and blood pressure and heart rate in rats during and after continuous administration. The results obtained demonstrated that inhaled ISBN can cause pronounced decreases in MAP and prominent increases in heart rate, and they are consistent with reports describing the acute effect of inhaled nitrites in humans (19,20). The short duration of action is also consistent with our previous report describing

the rapid systemic clearance of inhaled ISBN in rats (21).

Inhaled ISBN caused parallel reductions in systolic and diastolic blood pressure over the entire range of exposure concentrations and is consistent with the study by Perloff et al (22), who showed that inhalation of amyl nitrite in normal humans similarly decreased systolic and diastolic pressures about 30%. Thadani et al (23) studied the hemodynamic effects of oral isosorbide dinitrate (ISDN, an organic nitrate) in humans with angina pectoris and found that the decrease in systolic and diastolic pressure following ISDN administration was not parallel, with the percentage reduction of systolic pressure approximately twice that of diastolic pressure (approximate reduction: 35% for systolic; 19% for diastolic). These results indicate that organic nitrates and organic nitrites produce dissimilar pharmacological effects in humans.

Following the termination of inhaled ISBN, blood pressure returned to pre-inhalation values in about 10 to 15 minutes, suggesting that there was no apparent hemodynamic rebound from abrupt drug withdrawal. In contrast, rebound has been observed following withdrawal of NTG in a variety of hemodynamic parameters, including pulmonary wedge pressure, MAP, systemic vascular resistance, and left ventricular end diastolic pressure (LVEDP) (14,24). The mechanism of this rebound effect is believed to arise from unopposed neurohormonal vasoconstriction because of the rapid elimination of NTG (25) and may increase the incidence of ischemic episodes during the "dose-off" phase of NTG therapy (26). Bauer et al (15) showed that abrupt NTG withdrawal in rats causes rebound elevations of left ventricular end diastolic pressure to about 25% above baseline values, but this effect was completely avoided by graded NTG withdrawal. These data suggested that NTG-induced hemodynamic rebound is related to its rapid elimination and the concomitant production of physiological or biochemical counter-regulation (27,28). Despite the rapid clearance of ISBN (systemic half-life of about 1.5 minutes) (21), no hemodynamic rebound occurs with this vasodilator, suggesting that organic nitrites, in contrast to organic nitrates, do not activate counterregulatory effects within the exposure period.

The apparent steady-state blood levels of ISBN during inhalation were linearly related to the inhaled dose (inset of Figure 4B), suggesting that the clearance of ISBN was not changed over the 50-fold range of exposure concentrations administered in this study. We reported recently that the apparent bioavailability of inhaled ISBN was about 43% at doses of 309 and 907 ppm (21). The hemodynamic effects caused by ISBN were sustained over the 1-hour study period without apparent development of tolerance, another phenomenon that has been associated with organic nitrate administration. Bauer et al (14) compared LVEDP effects and tolerance properties of NTG versus infused ISBN and ISAN, and showed that the hemodynamic parameters of NTG returned to baseline within 10 hours while the organic nitrites maintained their effect even when infusions were carried out to 22 hours. Our findings that inhaled ISBN can maintain the decrease in MAP throughout the exposure regimen are consistent with those of Bauer et al and provide further evidence that organic nitrites are not apparently tolerance-producing, with 2 different routes of administration (infusion and inhalation) and using different hemodynamic parameters (LVEDP and MAP) as markers.

ISBN-induced increase in heart rate was sustained over the dosing interval and fell to pre-inhalation values at about the same rate as the blood pressure, suggesting that the rise in heart rate may be attributable to a reflex compensatory mechanism to increase blood pressure.

A simple sigmoid Emax model can be used to describe the relationship between steady-state blood ISBN concentrations and MAP effects, with an apparent EC_{50} of about 0.51 μM and a Hill coefficient of 1.2 (Figure 5). Bauer et al conducted in vitro isolated blood vessel experiments and determined that the EC_{50} and Hill coefficients for vascular relaxation of ISAN were approximately 0.82 μM and 1.3 μM, respectively (14). These results appear to be quantitatively similar to our present findings.

In addition to their different pharmacological properties compared with organic nitrates, as previously discussed, the organic nitrites may also possess certain potential advantages against other therapeutic NO donors. Inhaled NO (5 to 100 ppm) is used commonly as a selective pulmonary vasodilator for the treatment of diseases such as persistent pulmonary hypertension of the newborn and adult respiratory distress syndrome. We showed that at lower exposure levels (20-25 ppm), inhaled ISBN had little effect on blood pressure. In humans, it has been shown that inhaling high concentrations of ISAN can decrease pulmonary vascular resistance by 33% (29). If inhalation of ISBN at low concentrations can decrease pulmonary pressure without affecting systemic vascular tone, then organic nitrites may have some utility in the treatment of diseases associated with pulmonary hypertension. ISBN may also be a useful agent during surgery and hypertensive emergencies to control blood pressure because of its following favorable properties: a rapid hypotensive effect, no apparent rebound, and a short biological half-life. These composite PK/PD characteristics would ensure a rapid offset of action in the event of a severe reduction in blood pressure. SNP has been the NO donor of choice as an acute hypotensive agent. However, it produces cyanide ions upon metabolism and thus causes untoward side effects and toxicity upon prolonged administration. Co-infusion of thiosulfate is required during SNP infusion to protect the patient from the harmful effects of cyanide toxicity.

In summary, we have carried out a detailed examination of the concentration-effect relationship of inhaled ISBN in conscious rats with regard to blood pressure and heart rate. Data obtained from this study indicate that inhaled ISBN at various exposure levels produces a profound effect on the cardiovascular system, causing almost instantaneous decreases in blood pressure. The MAP effect appears to be related via a sigmoid E_{max} model to ISBN blood concentration. The characterization of the pharmacokinetic/pharmacodynamic actions of ISBN should enhance our understanding of this volatile agent, either as a therapeutic entity or as a drug of abuse.

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